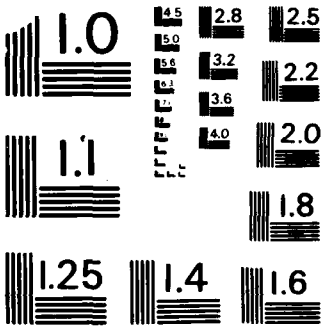
AD-A169 814 PHARMACOLOGY OF PERIODONTAL DISEASE(U) UNIVERSITY OF HEALTH SCIENCES/CHICAGO MEDICAL SCHOOL DEPT OF PHARMACOLOGY S F HOFF 23 JUN 86 N00014-84-K-0562 1/1

UNCLASSIFIED F/G 6/15 NL



MICROCOPY RESOLUTION TEST CHART

NATIONAL BUREAU OF STANDARDS - 1963 - A

(12)

University of Health Sciences/The Chicago Medical School

Department of Pharmacology

3333 Green Bay Road
North Chicago, Illinois 60064 312.578.3279

6-23-86

E.B. Hancock, CAPT, DC, USN, (Code 408)
Naval Medical Research and Development Command
Naval Medical Command
National Capital Region
Bethesda, MD 20814-5044

RE: Annual Letter Report
ONC Contract #N00014-84-K-0562
"Pharmacology of Periodontal Disease"

Dear Capt. Hancock:

The following is the annual report for the second year of funding on my ONR contract. I have modified and justified the budget request for the third year of funding, as we discussed previously. If you require additional information or clarification please do not hesitate to contact me. Thanks again for your continued interest and support of this project.

Sincerely,

Steven F. Hoff, Ph.D.

6-23-86

E.B. Hancock, CAPT, DC, USN, (Code 408)
Naval Medical Research and Development Command
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Bethesda, MD 20814-5044

Annual Letter Report
ONR Contract #N00014-84-K-0562
"Pharmacology of Periodontal Disease"
Steven F. Hoff, Ph.D. (Principal Investigator)

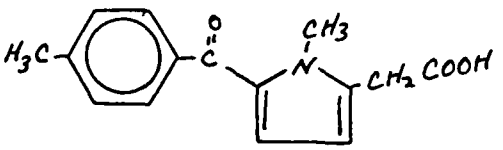
A. Progress Report:

This research pertains to identifying antibacterial and anti-inflammatory agents, used individually or in combination, which are appropriate for preventing the exacerbation of acute periodontal disease under field conditions.

During the second year of ~~our~~ the contract ~~we~~ research has have continued ~~our~~ studies using the human polymorphonuclear leukocyte (PMN) as a model system. One of the assays ~~we had~~ planned to used for these drug evaluations (Phagocytosis Assay) has demonstrated an excessive amount of variability ~~in our hands, and we have discarded~~ that assay has been discarded. In its place we have added a very sensitive assay for PMN Superoxide production, which is one of the primary mechanisms by which PMNs destroy bacteria. Excessive production of superoxide radicals may also cause damage to normal healthy tissues.

Some older antibacterial and anti-inflammatory compounds have been examined with regard to our objectives, and we have focused on more recently marketed compounds.

TOLMETIN



This non-steroidal anti-inflammatory drug (NSAID) has proven to be unusual in one way from other NSAIDs in that it significantly stimulates rather than inhibits PMN chemotaxis (Figure 1) (ANOVA $F=10.828$, df 3, $p< .01$). The Tukey-HSD procedure for multiple ranges shows this effect to be significant from control ($p< .05$) at the 50 ug/ml drug concentration. This is very clearly a dose-dependent process, which we have demonstrated occurs at drug concentrations found under therapeutic conditions. In addition Tolmetin causes a significant dose-dependent reduction in the release of beta-glucuronidase (dot filled bars) and lysozyme (hatched bars) from azurophilic and specific granules respectively (ANOVA $p<.01$ for both enzymes) (Figure 2). The Tukey HSD procedure

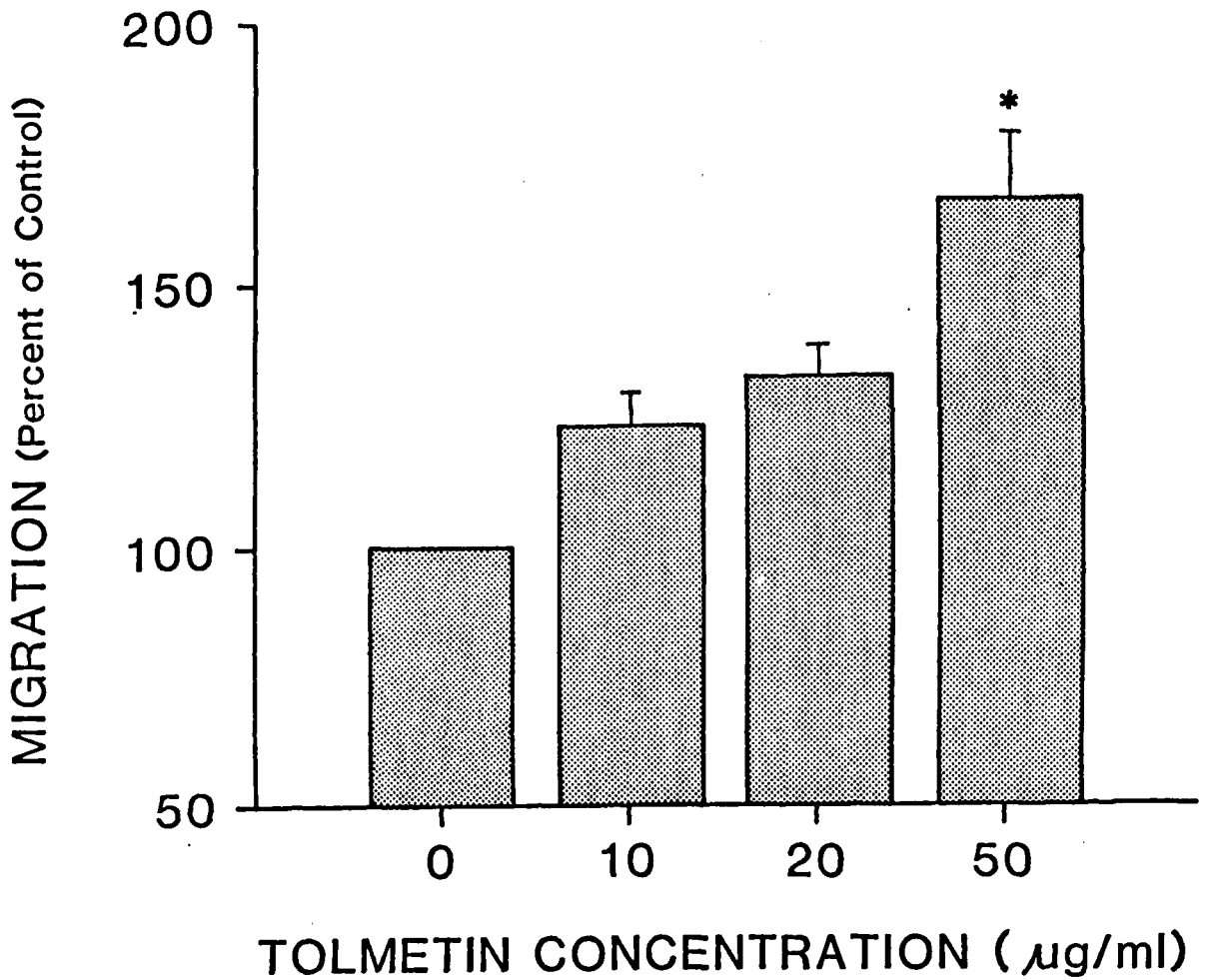


FIGURE 1. Effect of Tolmetin on PMN chemotaxis.
* $p < .05$ vs control (no drug)

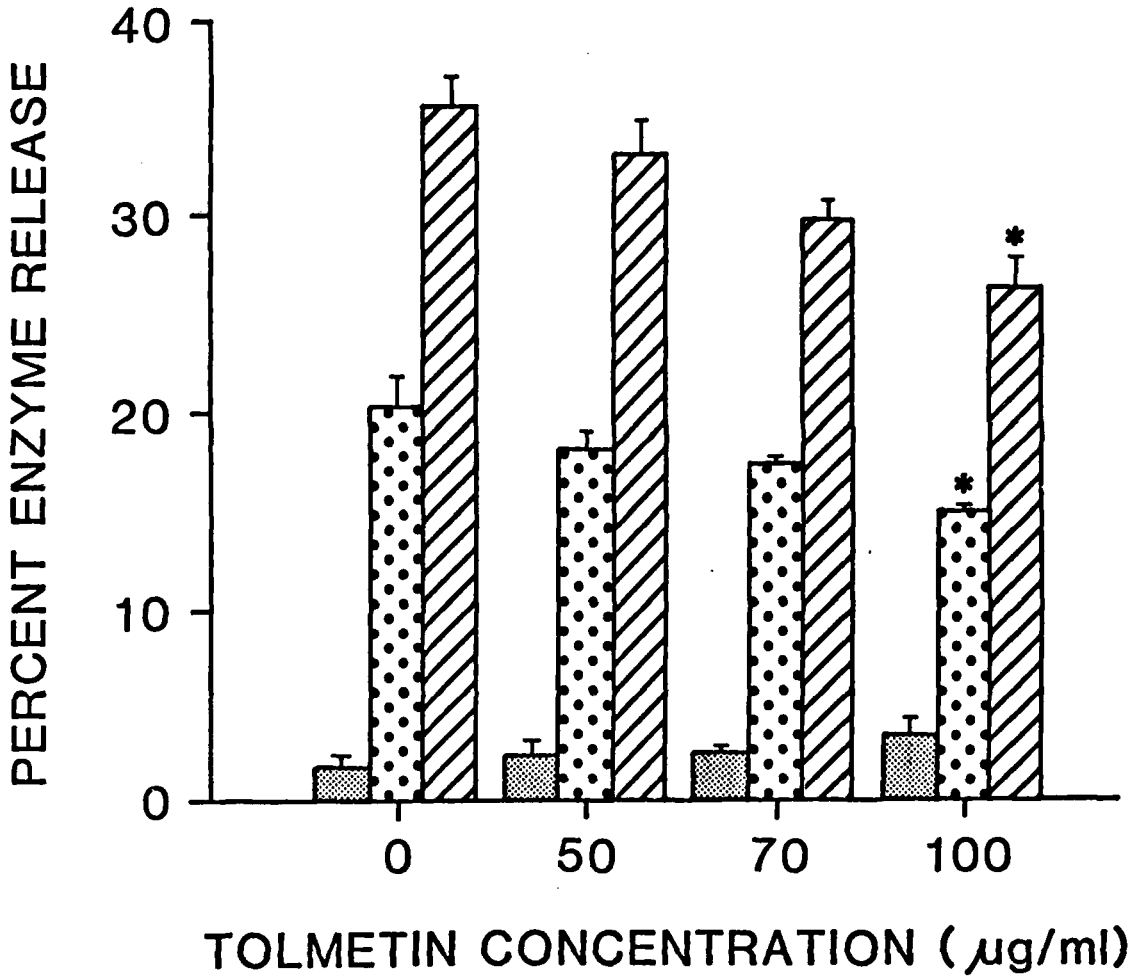
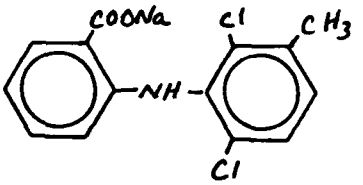


FIGURE 2. Effect of Tolmetin on PMN degranulation
Stippled bars=LDH activity (Lactate dehydrogenase)
Dotted bars=Beta-glucuronidase activity
Hatched bars=Lysozyme activity
* $p < .05$

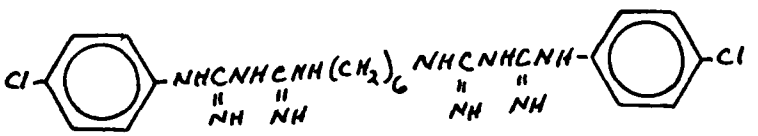
specifies that the effect is significant ($p<.05$) at the 100 ug/ml drug concentration. Cell death does not significantly increase with larger doses as indicated by the steady lactate dehydrogenase (LDH) levels (solid bars in Figure 2). The production of superoxide radials is also significantly inhibited by Tolmetin (Figure 3) (ANOVA $F=22.191$, df3, $p<.01$), with the effect significant at all drug concentrations (Tukey-HSD, $p<.05$). Our work on Tolmetin was presented at the 1986 FASEB meeting in St. Louis (see attached abstract).

MECLOFENAMATE



This NSAID is somewhat different from Tolmetin and appears to be much more potent in our assays. For example, Meclofenamate causes a significant dose-dependent reduction in chemotaxis by PMNs (Figure 4) (ANOVA, $F=133.979$, df4, $p<.0001$), with complete inhibition at 20 ug/ml. The Tukey-HSD procedure reveals that the drug effect is significant at the 5, 10 and 20 ug/ml concentrations ($p<.05$). Meclofenamate also causes a pronounced reduction in the release of beta-glucuronidase (ANOVA $F=29.293$, df3, $p<.0001$) and lysozyme (ANOVA $F=38.622$, df2, $p<.0001$), which is significant at all three drug concentrations (Tukey-HSD, $p<.05$) (Figure 5). Meclofenamate does not cause any significant increase in cell death under our experimental conditions (ANOVA, $F=2.875$, df3, $p>.1$) (Figure 5). Superoxide production is also markedly inhibited (Figure 6) (ANOVA $F=12.707$, df3, $p<.005$), and this is ignificant at the 20 and 50 ug/ml drug concentrations (Tukey-HSD, $p<.05$).

CHLORHEXIDINE



Chlorhexidine is a very potent antibacterial, which is applied topically. As with most NSAIDs, this antibacterial agent also caused a significant dose-dependent reduction in chemotaxis (Figure 7) (ANOVA $F=24.519$, df3, $p<.0002$), which was significant at the 15 and 25 ug/ml drug concentrations (Tukey-HSD, $p<.05$). Chlorhexidine did NOT alter the stimulated release of beta-glucuronidase (ANOVA $F=0.157$, df3, $p>.9$) or lysozyme (ANOVA $F=0.073$, df3, $p>.9$) (Figure

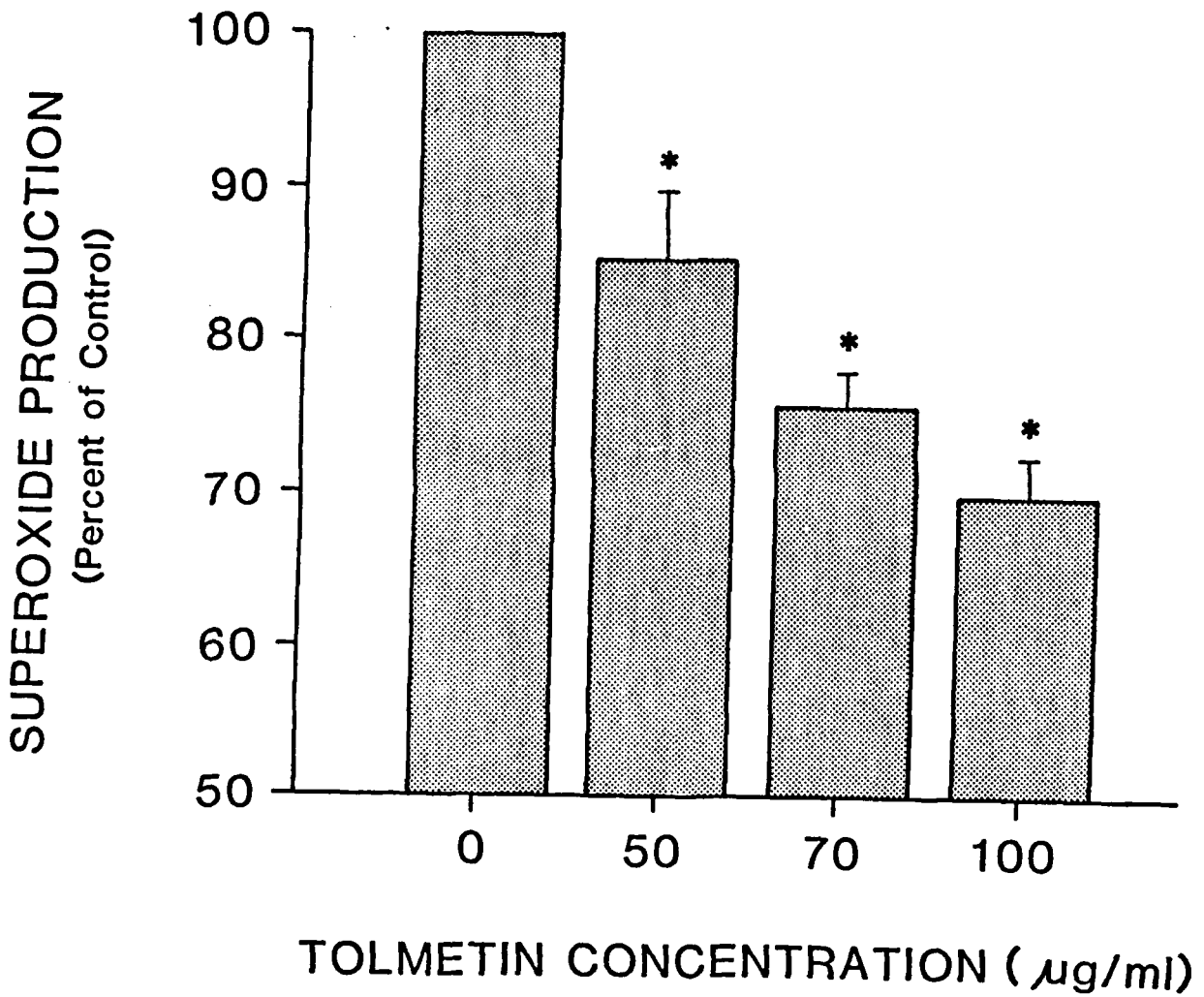


FIGURE 3. Effect of Tolmetin on superoxide production in PMNs
*p < .05

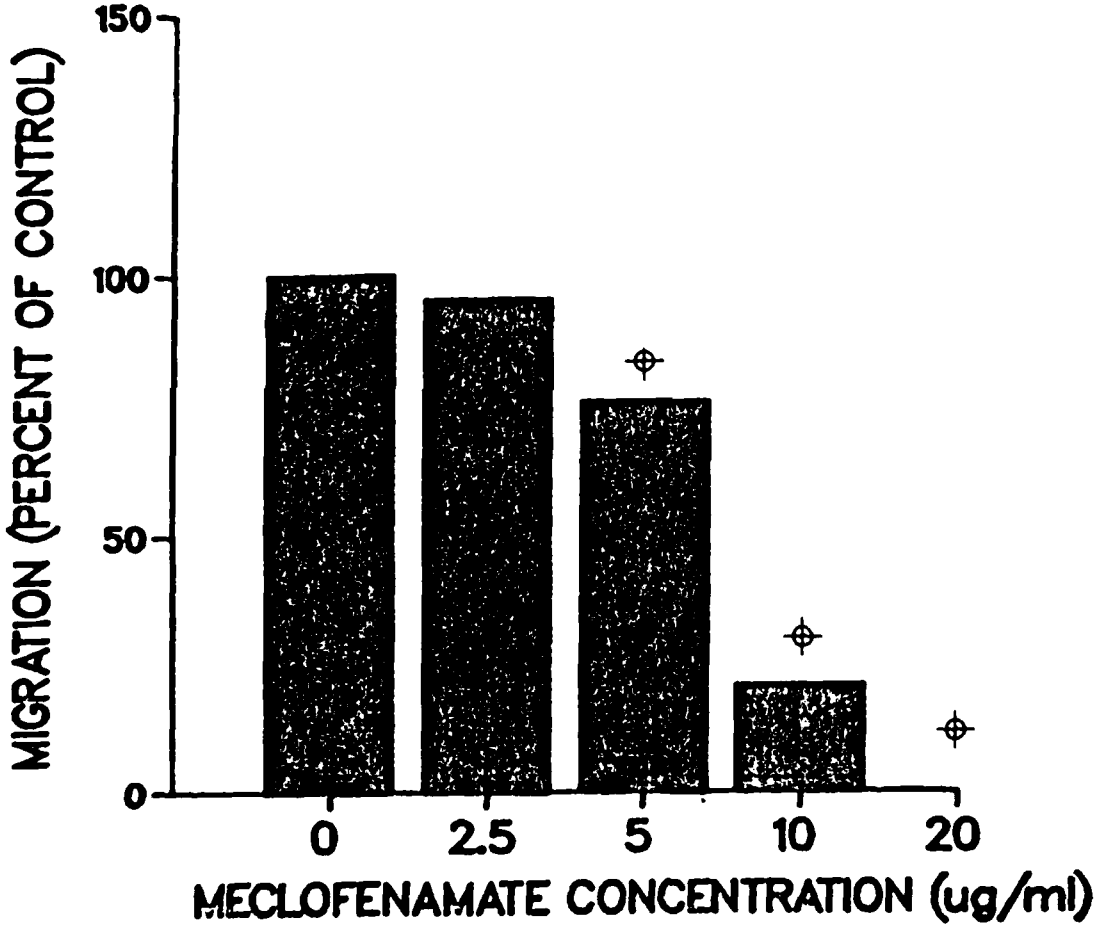


FIGURE 4. Effect of Meclofenamate on PMN chemotaxis.
⊕ $p < .05$

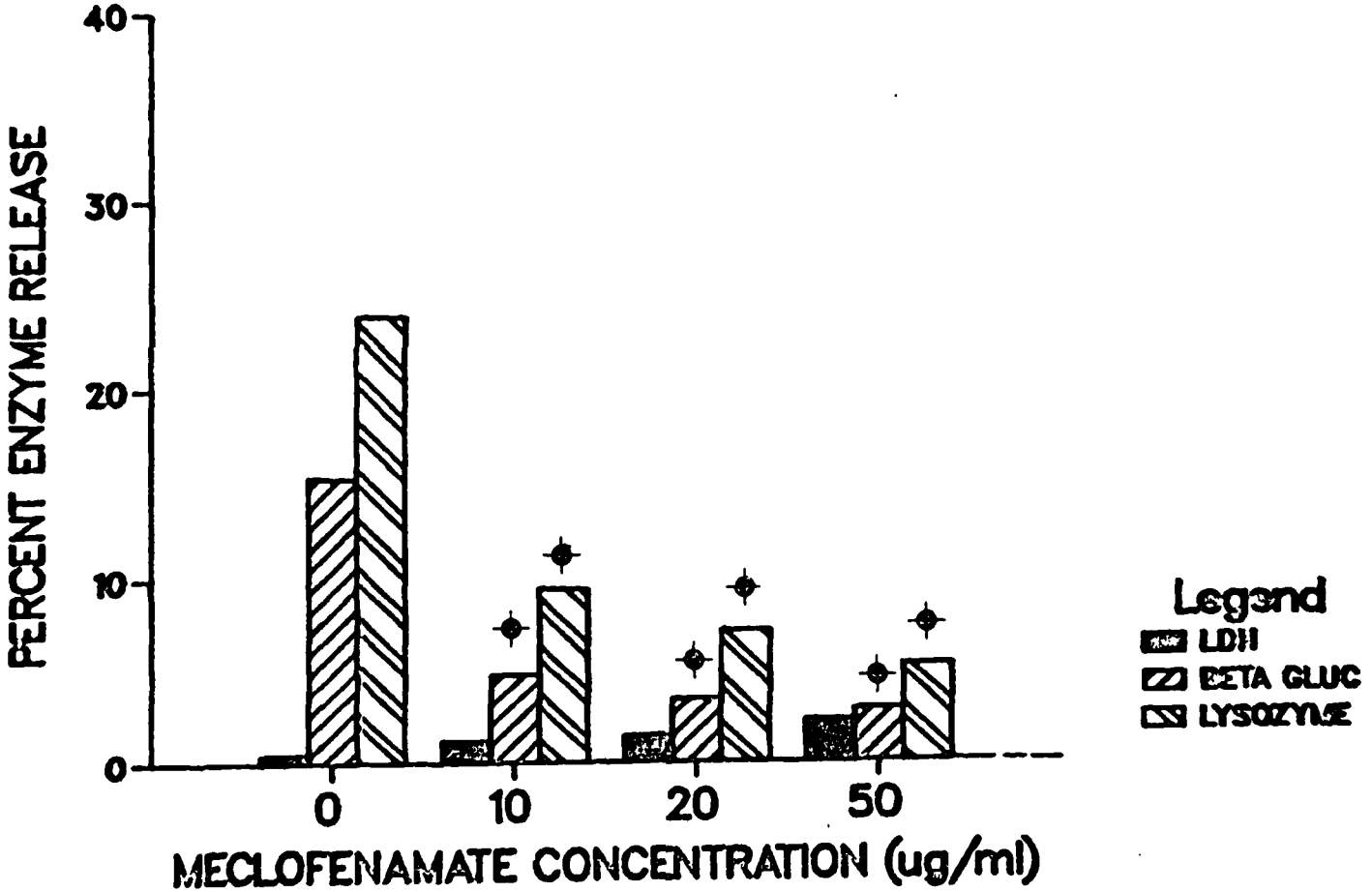


FIGURE 5. Effect of Meclofenamate on PMN degranulation.

✦ $p < .05$

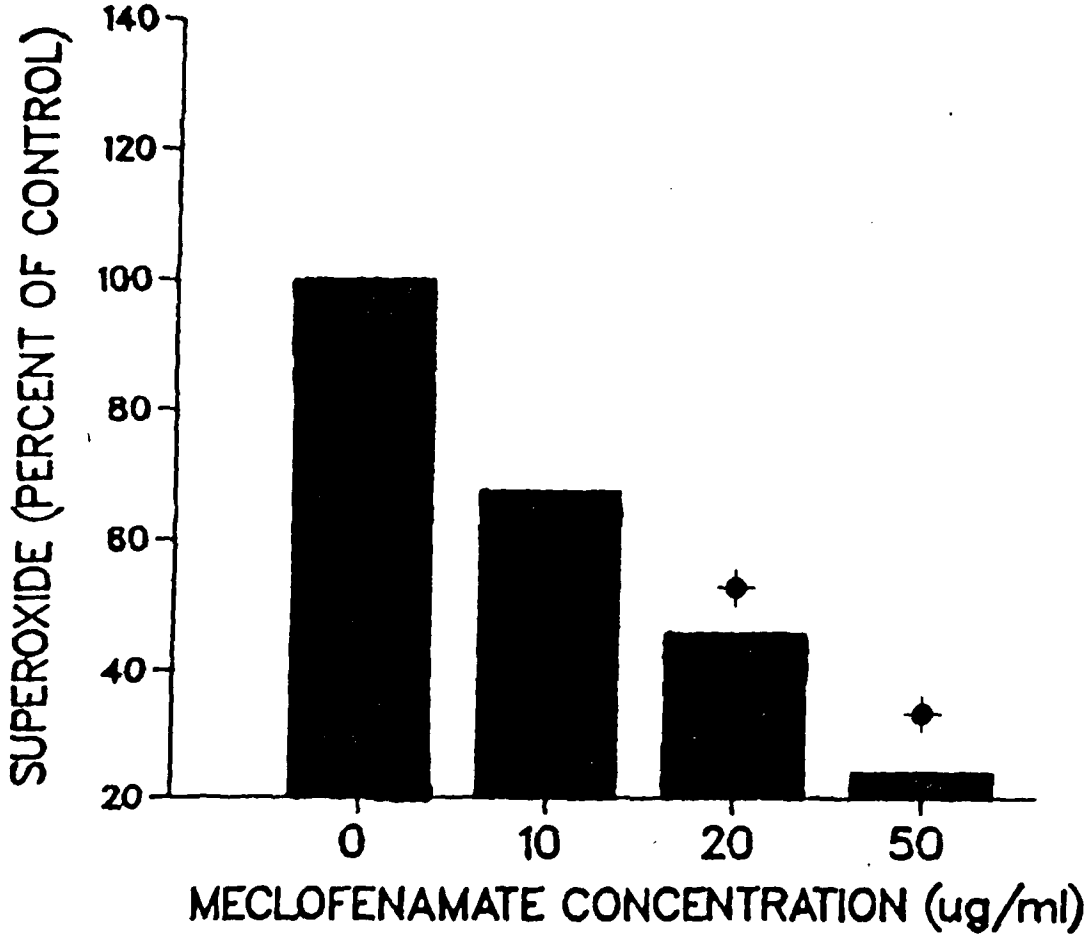


FIGURE 6. Effect of Meclofenamate on superoxide production by PMNs.

✦ $p < .05$

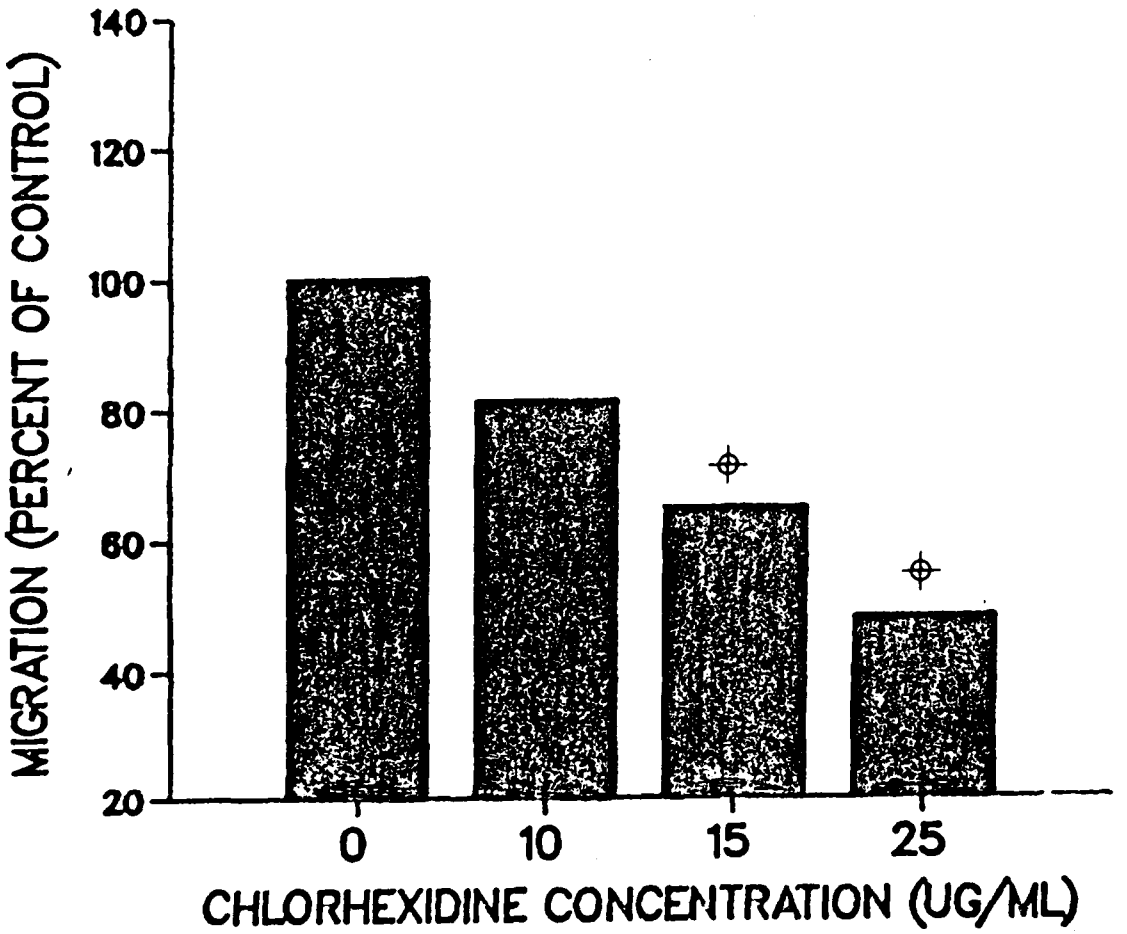
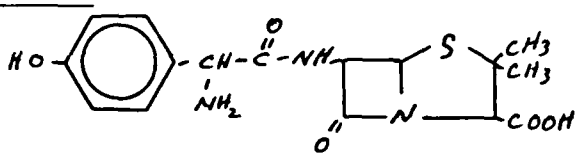


FIGURE 7. Effect of Chlorhexidine on PMN chemotaxis.

⊕ $p < 05$

8), eventhough a significant increase (ANOVA F=6.510, df3, p<.02) in the amount of cell death occured at the 100ug/ml drug concentration (Tukey-HSD, p<.05) (Figure 8). An interesting effect was observed, where a significant increase (ANOVA F=9.532, df3, p<.01) in the production of superoxide radicals occured in the presence of drug concentrations non-lethal to the PMNs (50ug/ml, Tukey-HSD, p<.05) (Figure 9).

AMOXICILLIN



Amoxicillin is a broad spectrum antibiotic from the penicillin family. It is beta-lactamase sensitive, so that its use in many staphylococcal infections is limited. However, when combined with a newly marketed compound, Clavulanic acid, which inhibits beta-lactamases, the spectrum of Amoxicillin is greatly extended. Though our data is not complete enough for statistical analysis at this time, it appears that Amoxicillin has no effect on chemotaxis, enyzme release or superoxide production (Table I).

TABLE I. EFFECTS OF AMOXICILLIN ON PMN FUNCTIONS

DRUG CONCENTRATION:	CHEMOTAXIS	ENZYME RELEASE LYSOZYME	ENZYME RELEASE BETA-GLUC	SUPEROXIDE PRODUCTION
CONTROL	100%*	35.1%	24.4%	100%
5 ug/ml	101.5%	-----	-----	-----
10ug/ml	108.8%	32.6%	22.6%	105.2%
20ug/ml	105.1%	33.9%	24.8%	103.0%
50ug/ml	104.1%	31.6%	25.4%	109.1%
100ug/ml	111.5%	31.8%	26.7%	------

* data given as percent of control

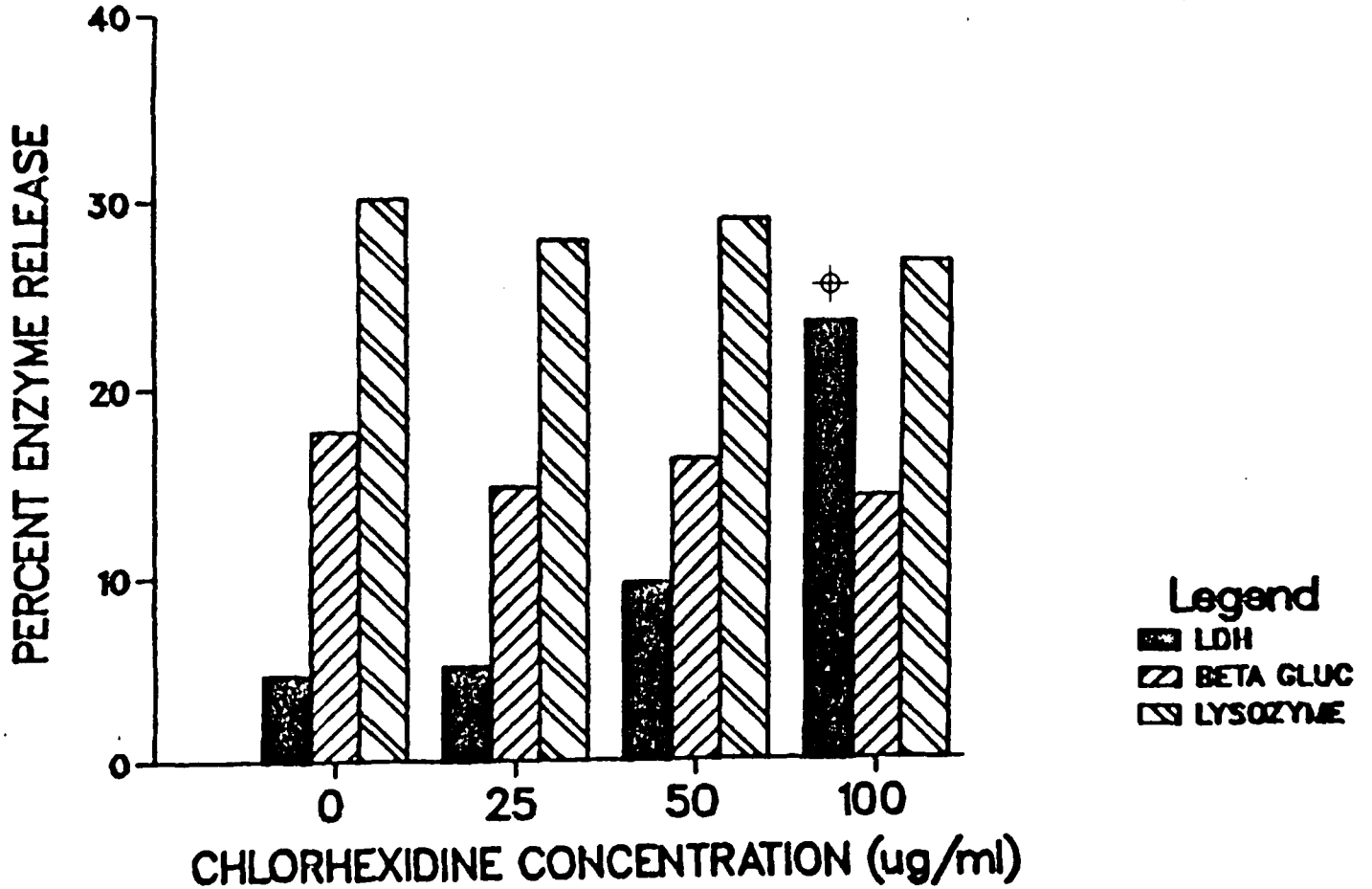


FIGURE 8. Effect of Chlorhexidine on PMN degranulation

⊕ $p < .05$

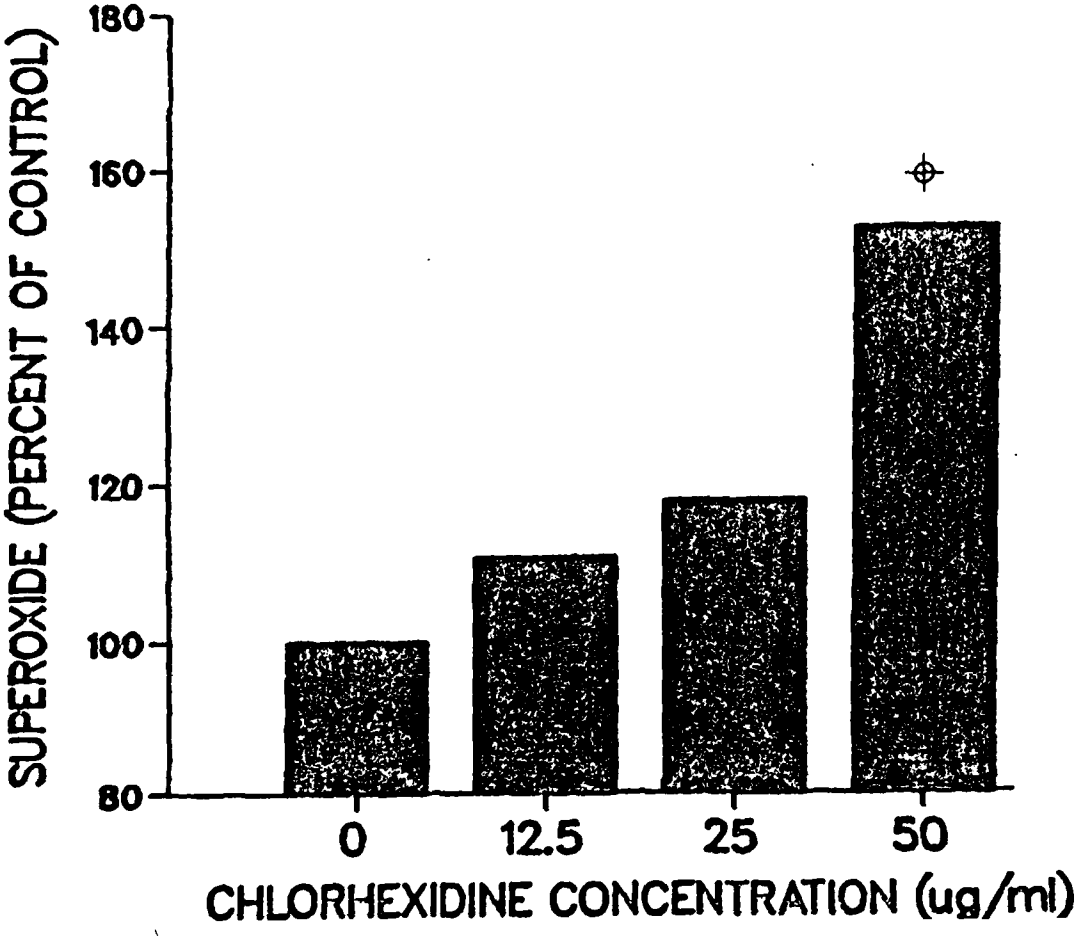
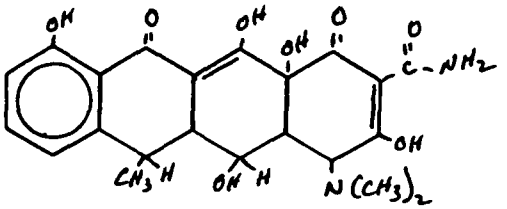


FIGURE 9. Effect of Chlorhexidine on superoxide production by PMNs.

⊕ $p < .05$

DOXYCYCLINE



Doxycycline is one of the broad spectrum tetracycline antibiotics. We have just begun to evaluate this compound in our assay procedures. At a concentration of 10ug/ml, doxycycline appears to inhibit superoxide production by about 50%. At concentrations above this (20 and 50 ug/ml), the inhibition appears erratic and this may be caused by a direct inhibition of superoxide dismutase, which is in our reaction mixture.

Several additional compounds with relevance to our studies have been examined in the literature (Table II). To date, it appears that Meclofenamate is the most potent anti-inflammatory agent for use in acute periodontal inflammation, while chlorhexidine may prove to be very useful as an antibacterial agent. Chlorhexidine is available for topical use in the United States, but not for oral indications. This application is being used in Europe, and approval here should be forthcoming.

TABLE II. SUMMARY OF DRUG EFFECTS

DRUG:	CHEMOTAXIS	ENZYME RELEASE BETA-GLUC	ENZYME RELEASE LYSOZYME	SUPEROXIDE PRODUCTION
TOLMETIN	I	D	D	D
MECLOFENAMATE	D	D	D	D
CHLORHEXIDINE	D	ne	ne	I
DOXYCYCLINE	-	-	-	D
AMOXICILLIN	ne	ne	ne	ne
(From the literature)				
INDOMETHACIN	D	D	D	D
NAPROXEN	D	D	D	NR
IBUPROFEN	D	D	D	D
TETRACYCLINES	D	NR	NR	NR
CLINDAMYCIN	±	D	D	D

I=increased; D=decreased; ne=no effect; NR= not reported
±=conflicting reports; -=not done yet

B. WORK PLAN FOR THIRD YEAR:

During the coming year we will evaluate the effects of appropriate combinations of NSAIDs and antibacterials on PMN functions either in culture alone or co-cultured with human periodontal fibroblasts. This will entail the biochemical and morphological examination of PMN and fibroblast interactions in culture under FMLP stimulated and unstimulated conditions, in the presence or absence of the different therapeutic agents. Once these experiments are completed, we will compile a complete report of our experimental protocols, assay procedures, experimental findings, and recommendations.

Third Year Milestone:

We expect that the work accomplished in the final year of this contract will result in a useful recommendation for the use of combined agents in the prevention of chronic periodontal disease. This study should provide an excellent opportunity for transition to advanced development (6.3) of drug delivery systems for field use by independent duty corpsmen.

C. BUDGET FOR YEAR THREE:

	ORIGINAL BUDGET	REQUESTED BUDGET
PERSONNEL	$40,094	$64,350
EQUIPMENT	-0-	-0-
SUPPLIES	14,300	14,000
TRAVEL	2,500	2,000
OTHER	6,800	4,000
INDIRECT COSTS	36,306	48,080
TOTAL COSTS:	100,000	132,430

Equipment List and Justification:

No new major pieces of equipment are required during the third year of our contract.

Third Year Budget Changes and Justification:

Our original contract proposal had expected our experimental workload to decrease significantly as we entered the third year of the contract and allow us to eliminate one of the personnel positions. However, during the first year, we modified our protocols and experimental model system in order to better achieve our objective (ie. to identify possible combinations of anti-inflammatory and antibacterial agents which could prove useful in the prevention of chronic periodontal disease under field conditions). We are very satisfied that our modified approach to

this task is appropriate and far more objective and reliable, however these modifications have in turn altered the distribution of our workload over the contract period. In our original contract proposal, we projected a strong beginning to our evaluation of drug combinations during the second year of the contract. With our modified experimental protocols, we are just going to finish the analysis of the single agents during the second year. To ensure our ability to complete the contract goals, I strongly believe that the retention of the full technical staff is imperative, and for this reason I have requested additional funds in the PERSONNEL category along with a concommitant addition to the INDIRECT COST category to cover University expenses. The indirect cost still remains at the agreed upon percentage of total direct costs less equipment. Additionally, I have lowered the original funds requested in the TRAVEL and OTHER categories to equal that used during the second year. This level of funding has proven to be totally adequate for our requirements. The requested funds for SUPPLIES has not changed significantly from the original proposal. A more detailed breakdown of the budget is attached at the end of this progress report.

PRINCIPAL INVESTIGATOR/PROGRAM DIRECTOR: STEVEN F. HOFF, Ph.D.

DETAILED BUDGET FOR **12 MONTH BUDGET PERIOD** | FROM 10-1-86 | THROUGH 9-30-87

DOLLAR AMOUNT REQUESTED *(Omit cents)*

PERSONNEL *(Applicant organization only)* NAME	POSITION TITLE	TIME/EFFORT %	Hours per Week	SALARY	FRINGE BENEFITS 20%	TOTALS
Hoff, Steven F.	Principal Investigator	10		3440	690	4138
Shelly, John	ResearchTechnician	100		17496	3499	20995
Jacoby, Larry	" "	100		16956	3391	20347
Walker, Shirley	" "	100		15725	3145	18870
	SUBTOTALS →			53625	10725	64350

CONSULTANT COSTS		-0-
EQUIPMENT *(Itemize)*		-0-
SUPPLIES *(Itemize by category)* Tissue culture media, glassware, chemicals, CO_2, ($6500); Assay biochemicals ($3500); Microscopy chemicals, fixatives, stains, photosupplies ($2000); Disposables, general lab chemicals, glassware ($2000)		14000
TRAVEL	DOMESTIC	2000
	FOREIGN	
PATIENT CARE COSTS	INPATIENT	
	OUTPATIENT	
ALTERATIONS AND RENOVATIONS *(Itemize by category)*		-0-
CONSORTIUM/CONTRACTUAL COSTS		-0-
OTHER EXPENSES *(Itemize by category)* Blood donations ($600); Books, library services ($400); Page charges, illustrations, reprints, typing ($1000); EM facility recharges ($2000)		4000
INDIRECT COSTS:		48080
TOTAL COSTS →		$ 132430

1046

EFFECT OF ADRENALIN ON MEMBRANE POTENTIAL CHANGES AND THE PROLIFERATION RESPONSE OF MURINE T LYMPHOCYTES. J.F. Beaudin and R. Gorczynski, Ontario Cancer Institute, Toronto, Canada

The presence of adrenergic receptors on T lymphocytes has been well documented. The number of these receptors as well as their Kd values is comparable to that of other tissues.

Normal mouse splenic T cells or spleen cells hyperimmunized to keyhole limpet hemocyanin, (KLH), exposed to brief pulses of pharmacological doses of adrenalin, show suppression of the proliferative response when subsequently exposed to mitogenic doses of ConA or KLH, respectively, for 72 hours.

These observations have been correlated with membrane potential changes where exposure of the normal mouse splenic T cells to ConA or the hyperimmunized cells to KLH hypopolarizes the cell membrane. In contrast, adrenalin hyperpolarizes the cell membrane and causes reversal of the membrane changes seen with ConA or KLH.

FEDERATION PROCEEDINGS 45(3): 3-1-86

ANTIINFLAMMATORY AGENTS (1047–1050)

1047

ANTI-INFLAMMATORY AND IMMUNOREGULATORY ACTIVITY OF SKAF 86002: A DUAL INHIBITOR OF ARACHIDONATE METABOLISM. M. J. DiMartino, W. Johnson, B. Berkowitz and N. Hanna, Smith Kline & French Laboratories, Philadelphia, PA 19101.

SKAF 86002 [6-(4-fluorophenyl) 2,3-dihydro-5-(4-pyridinyl) imidazo (2,1-b) thiazole] administered orally to rats, inhibited the development of carrageenan-induced edema, immune and non-immune mediated inflammation of adjuvant-induced arthritis (AA) and decreased established inflammation of AA and collagen type II-induced arthritis. SKAF 86002 produced dose related analgesia in the phenylquinone-induced abdominal constriction mouse assay, which was not reversed by naltrexone, a narcotic antagonist. In addition, SKAF 86002 produced anti-inflammatory activity in rat paw edema assays which are insensitive to cyclooxygenase inhibitors including: ongoing (established) carrageenan edema and edema induced by arachidonic acid and platelet activating factor. Moreover, SKAF 86002 inhibited the elicitation of immune-mediated inflammatory responses to purified protein derivative and the development of hindleg paralysis associated with experimental allergic encephalomyelitis. In summary, SKAF 86002, a dual inhibitor of arachidonate metabolism, is an orally effective antiarthritic/analgetic compound possessing corticosteroid-like anti-inflammatory and immunoregulatory properties.

1048

NE-19550, AN ANTI-INFLAMMATORY/ANALGESIC AGENT WITH A NOVEL MECHANISM. L. M. Brand, K. L. Skare, M. E. Loomans, M. L. Skoglund, H. H. Tai, C. Chiabrando, and R. Fanelli (SPON: J. T. Rotruck). The Procter & Gamble Company, P.O. Box 39175, Cincinnati, OH 45247; Dept. of Pharmacognosy, Univ. of Kentucky, Lexington, KY; Mario Negri Institute, Milan, ITALY.

NE-19550 (N-(3-methoxy-4-hydroxybenzyl)-oleamide) is an analog of capsaicin that has been shown to possess analgesic and anti-inflammatory activities in several animal tests. Capsaicin is an anti-oxidant that has been reported to inhibit cyclooxygenase activity in vitro (Prostaglandins 20: 209 (1980)). To elucidate the role of arachidonic acid metabolism in the anti-inflammatory mechanism of action of NE-19550, the inhibition of cyclooxygenase and lipoxygenase enzymes by NE-19550 was examined. In vitro, NE-19550 inhibited canine gastric cell PGE2 synthesis (Ki=0.5 μM) and also the 5-lipoxygenase from RBL-2H3 cells (IC50=0.9 μM). The non-analgesic trans-isomer of NE-19550 was not inhibitory toward these enzymes. Other enzymes involved in arachidonic acid metabolism (i.e., thromboxane synthetase, prostacyclin synthetase, and 12- or 15-lipoxygenase) were unaffected by either isomer. In vivo, analgesic/anti-inflammatory doses of NE-19550 did not suppress unstimulated prostaglandin or thromboxane accumulation by either platelets, brain, kidney, or lung. NE-19550 also did not inhibit the net release of LTB4 by intact leukocytes. It is concluded that the action of NE-19550 in vivo does not involve NSAID-like inhibition of prostanoid synthesis.

1049

TOLMETIN MODULATES FORMYL-PEPTIDE STIMULATED HUMAN NEUTROPHIL FUNCTIONS. J. Shelly, L. Jacoby, S. Walker, and S. Hoff (SPON: A. Vazquez). Department of Pharmacology, The Chicago Medical School, North Chicago, IL 60064.

Studies were conducted to determine how Tolmetin, a non-steroidal antiinflammatory drug (NSAID), would affect several neutrophil functions associated with the inflammatory response. Therapeutic concentrations of Tolmetin (50-100μg/ml) were found to increase significantly ($p<0.01$) FMP induced chemotaxis of human neutrophils under agarose in a dose dependent manner, and FMLP stimulated neutrophils demonstrated a significant ($p<0.01$) dose dependent decrease in lysosomal enzyme release (both specific and azurophilic granules) after Tolmetin treatment. Also, phagocytosis of latex beads and superoxide anion production were markedly reduced when compared to control stimulated neutrophils. Tolmetin differs in its actions from other NSAIDs, such as Indomethacin and Ibuprofen, in its ability to increase the chemotactic responsiveness of neutrophils. These data support the proposal that NSAIDs may differentially affect discreet neutrophil functions, and thus these drugs may have multiple but different sites of activity involved in their antiinflammatory actions.
(Supported by ONR Contract N1484K0562).

1050

INHIBITION OF LYMPHOCYTE PROLIFERATION AND INFLAMMATORY RESPONSES IN ADJUVANT-ARTHRITIC RATS BY ANTI-INFLAMMATORY DRUGS. George F. Seng and Barbara M. Bayer. Georgetown University, Washington D.C. 20007.

Administration of indomethacin to rats twice a day for a three day period resulted in a dose-dependent inhibition of splenic lymphocyte proliferation stimulated by phytohemagglutinin (PHA). The inhibition was accompanied by a decrease in the lymphocyte sensitivity and maximal response to PHA. Partial inhibition of the response was observed with 0.25 mg/kg and complete inhibition with doses exceeding 0.5 mg/kg of indomethacin. The inhibition of the PHA-response was also found to be time-dependent; 1 mg/kg for 1 day resulted in only a 30 percent inhibition whereas complete suppression occurred after 3 days of indomethacin administration. The magnitude and time-dependency of these changes were found to be highly correlated to the reduction of contralateral paw volumes of rats injected with adjuvant 14 days previously. Flufenamic acid (8 mg/kg), piroxicam (1 mg/kg) and phenylbutazone (30 mg/kg) also were found to completely inhibit the lymphocyte response and reduce paw volumes to that of controls. In contrast, acetaminophen (600 mg/kg) had little effect on either parameter. (NIH Grant AI20076)

END

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